PB86-167913

Offspring Mortality and Maternal Lung Pathology in Female Rats Fed Hexachlorobenzene

(U.S.) Environmental Protection Agency
Research Triangle Park, NC

1982

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TECHNICAL REPORT DATA *(Please read Instructions on the reverse before completing)*		
1. REPORT NO. EPA/600/J-82/016	2.	3. REC PB86-167913
4. TITLE AND SUBTITLE OFFSPRING MORTALITY AND MATERNAL LUNG PATHOLOGY IN FEMALE RATS FED HEXACHLOROBENZENE (Journal Version)		5. REPORT DATE 1982 6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) [1]Kirk T. Kitchin, Ralph E. Linder, Thomas M. Scotti, Debra Walsh, August O. Curley, and Dave Svendsgaard		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS [1]U.S. Environmental Protection Agency, Developmental Biology Division, Health Effects Research Laboratory, Research Triangle Park, NC 27711		10. PROGRAM ELEMENT NO. 11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS Health Effects Research Laboratory Office of Research and Development U.S. Environmental Protection Agency Research Triangle Park, NC 27711		13. TYPE OF REPORT AND PERIOD COVERED Journal Article 14. SPONSORING AGENCY CODE EPA - 600/11
15. SUPPLEMENTARY NOTES Toxicology, 23 (1982) 33-39 January No. 1 Elsevier/North-Holland Scentific Publishers Ltd.		

16. ABSTRACT

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17. KEY WORDS AND DOCUMENT ANALYSIS		
a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

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NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
RELEASE TO PUBLIC	UNCLASSIFIED	9
	20. SECURITY CLASS *(This page)* UNCLASSIFIED	22. PRICE

EPA/600/J-82/016
JOURNAL ARTICLE

Toxicology, 23 (1982) 33—39
Elsevier/North-Holland Scientific Publishers Ltd.

OFFSPRING MORTALITY AND MATERNAL LUNG PATHOLOGY IN FEMALE RATS FED HEXACHLOROBENZENE

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(Received September 10th, 1981)
(Accepted December 16th, 1981)

SUMMARY

Female Sprague–Dawley CD rats were fed 0, 60, 80, 100, 120 and 140 ppm hexachlorobenzene (HCB) continuously in the diet and 2 successive litters raised. These doses were selected to range from approximately the no observable effect level to lethality in suckling offspring of treated dams. In the F_{1a} generation, the 21-day mortality was 9.2, 19.8, 30.0, 45.4, 93.1 and 92.6% in offspring of dams fed 0, 60, 80, 100, 120 and 140 ppm HCB, respectively. In the F_{1b} generation, a similar mortality of 18.5, 21.5, 19.5, 45, 100 and 94.1% was observed at these 5 dose levels, respectively. The neonatal lethality observed was related to both maternal dose of HCB and the cumulative lactational exposure. Clinical signs of maternal toxicity were not observed and fertility and fecundity were unaffected. In the lungs of HCB treated dams, increased numbers of intraalveolar foamy histiocytes and hypertrophy and proliferation of the lining endothelial cells of pulmonary venules were observed. These microscopic findings of pulmonary effects of HCB confirmed previous findings of this laboratory.

INTRODUCTION

Hexachlorobenzene (HCB) is a registered fungicide, a toxic waste produced in the manufacture of chlorinated solvents and other compounds,

*To whom reprint requests should be addressed.
Abbreviation: HCB, hexachlorobenzene.
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0300-483X/82/0000—0000/$02.75

as well as a production contaminant in the pesticides dimethyl tetrachloroterephthalate and pentachloronitrobenzene [1]. HCB is not readily metabolized and excreted by mammals and accumulates in biological organisms and food chains [2]. The terminal phase half-life of HCB in female rat plasma, brain, fat and liver is 56, 101, 62 and 65 days, respectively [3]. HCB can be transferred to offspring by both the placental and lactational routes [4]. In rats, suckling offspring are especially vulnerable to HCB-induced mortality [5]. A mass poisoning of Turkish adults and children occurred in the 1950s due to the consumption of HCB containing fungicides [6].

In rats fed a constant level of HCB the majority of the late neonatal HCB body burden is from lactational exposure. Mendoza et al. [4] fed rats 80 ppm HCB and cross-fostered the offspring. At day 21 the neonatal brain levels of HCB were 1.3 and 40.6 μg/g for pups nursed by control and HCB treated foster mothers, respectively. The day 21 brain levels of control pups nursed by control or HCB treated foster dams was 0.12 and 36.4 μg/g HCB, respectively [4].

The objective of this study was to accurately determine the LD_{50} in the F_{1a} and F_{1b} offspring of HCB treated dams and to determine if a difference in mortality was present between the F_{1a} and F_{1b} generations due to substantial lactational transfer of HCB to the F_{1a} generation. Such a depletion of maternal body burden of toxicant could help explain interlitter differences in reproductive toxicity studies (e.g., more toxicity in F_{1a} than F_{1b}). In addition, maternal pulmonary tissue was evaluated for the presence of intraalveolar foamy histiocytes and hypertrophy and proliferation of the endothelial venules.

MATERIALS AND METHODS

Animals and treatments

Female Sprague—Dawley rats (CD) were obtained from Charles River and housed in a biocIean, laminar flow animal facility. The weanling rats (10 per treatment group) were fed 0, 60, 80, 100, 120, and 140 ppm HCB in the diet throughout the experiment. HCB (>99% purity) was purchased from BDH Chemicals, LTD, Poole, England. The HCB was ground to a fine powder with a mortar and pestle and blended with Purina rat chow to prepare a HCB premix. The HCB treatment diets were prepared by diluting the HCB premix with additional Purina rat chow in a large blender.

After 96 days on the treatment diet the female rats were individually mated with control male rats. The offspring were born in individual nestboxes with no culling of litters or equalization of litter size. The numbers of live and dead pups at birth were recorded. The number of surviving pups was determined on days 4, 9, 14 and 21, respectively, and the pup body weights were measured on days 1, 4 and 21. Females were bred for the second time 12 days after weaning or the death of the last pup.

Histology and HCB residue analysis

After the F_{1b} generation was weaned lungs were taken from dams for microscopic evaluation. At necropsy the lungs were infiltrated with 10% neutral buffered formalin, removed and immersed in the fixative. Hematoxylin and eosin staining of 7-μm sections was done by standard procedures. Day 21 rat pup brain HCB residues were analyzed by the method of Watts et al. [7], with the omission of the Florosil column which was used for cleanup and the derivitization to bis-isopropoxytetrachlorobenzene. A Tracor Model 222 gas chromatograph equipped with an electron capture detector was used to quantify HCB tissue residues. Tissue residue values were corrected for HCB recoveries which ranged between 80% and 98%.

Statistics

All statistics were done using the litter as the sampling unit. Standard error of the mean was used as a measure of variation. Weights were averaged litter weights and mortality was average percentages of dead pups per size of the litter. Jonckheere's Test [8] was used on both weights and mortality to test for a dose-response effect. Williams' test was used to identify the lowest dose that varied significantly from the control response [9]. Probit analysis was used on the birth to day 21 cumulative mortality to estimate the LD_{50} dose based on the log dose. In testing for a dose-related differential effect between generations, the mother was the sampling unit. The data of mothers that were not pregnant in the F_{1a} generation were excluded from the test.

RESULTS

No dose-related maternal toxicity, either by clinical observation, weight gain or lethality was noted in this study. At birth the fertility and litter size of HCB treated rats were not significantly different from control values in either the F_{1a} or F_{1b}. During the F_{1a} lactational period, substantial mortality occurred in suckling pups of HCB treated dams (Fig. 1). By day 21 of lactation the percent of dead pups per litter averaged 19, 30, 45, 94 and 92% in the 60, 80, 100, 120 and 140 ppm HCB groups, respectively, as compared to 11% of the controls. Most deaths occurred before the 14th day of lactation and the highest rate of mortality occurred between days 4 and 9. The mortality was significantly related to dose ($P < 0.001$) at days 4, 9, 14 and 21. The LD_{50} for day 21 cumulative mortality from birth was 100 ppm (95% confidence interval was 66–120 ppm) for the F_{1a} generation. The best fit to the data was found by using the unlogged dose and adjusting for control mortality.

In the F_{1b} generation the day 21 cumulative mortality was 19, 22, 20, 45, 100 and 94% at 0, 60, 80, 100, 120, and 140 ppm HCB, respectively. F_{1b} day 21 rat pups nursed by dams administered 60 ppm HCB in the diet exhibited brain levels of 53 ± 3.5 μg/g HCB ($N = 5$).

Considering the neonatal mortality of pups born to HCB treated rats,

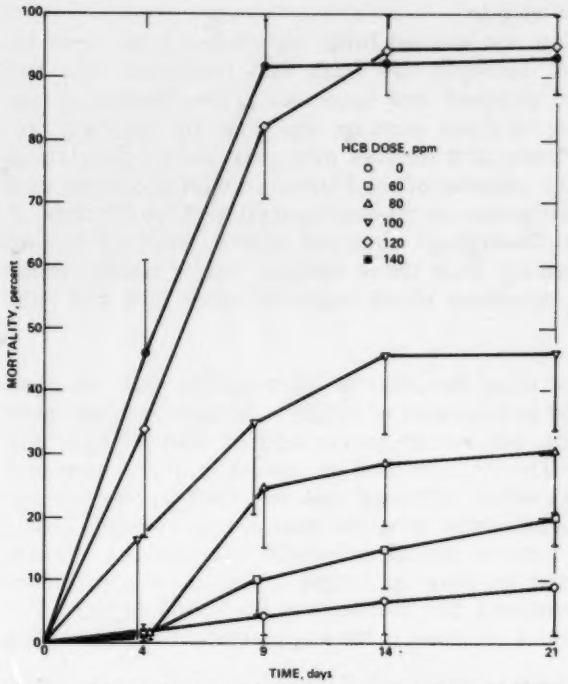

Fig. 1. Effect of maternal dietary concentration of HCB on neonatal rat pup mortality in the F_{1a} generation. The percent mortality and the S.E.M. are plotted vs. days after birth.

few significant decreases in birth weight and growth were observed (Table I). In both the F_{1a} and F_{1b} generations the pups born of dams fed 120 ppm or 140 ppm HCB had normal birth weights, but significantly lower (65—85% of controls) body weights by day 4. Since approximately 95% of the rat pups in those dose groups died by day 21 of lactation no meaningful statistical comparisons of body weight on day 21 can be made, however, the few surviving pups reached body weights comparable to controls. Control neonatal weight gain and litter size are known to be inversely related. At day 4, decreases in rat pups body weight were significantly related to the dose ($P < 0.01$). The 60 ppm and 80 ppm HCB treatment groups had the least pup mortality (18–30%) in this study. These 2 groups of pups had lower body weights on day 21 of lactation (79—89% of control values). Three of the 4 comparisons were significantly different by the Students' *t*-test [10], although these decrements in 21 day pup body weights were not significantly related to dose.

In the 60-ppm HCB treatment group the F_{1b} newborn pups had 35% less HCB body burden than the F_{1a} generation (Table II). Similarly the day 21 rat pup brain HCB levels were 22% lower in the F_{1b} than in the F_{1a}.

TABLE I

NEONATAL GROWTH DURING PARENTAL HEXACHLOROBENZENE ADMINISTRATION

Data is the mean ± S.E. of the mean of the indicated number of litters. Body weight is in grams.

Time	Generation	0 ppm	60 ppm	80 ppm	100 ppm	120 ppm	140 ppm	Jonckheere's dose related test
Day 1	F_{1a}	6.5 ± 0.2(6)	5.4 ± 0.2(6)[b]	6.1 ± 0.2(9)	6.4 ± 0.2(6)	6.2 ± 0.2(6)	6.2 ± 0.1(9)	NS
	F_{1b}	6.1 ± 0.1(6)	6.0 ± 0.2(6)	6.5 ± 0.2(9)	6.5 ± 0.3(6)	6.2 ± 0.1(6)	6.1 ± 0.2(9)	NS
Day 4	F_{1a}	9.4 ± 0.3(6)	9.3 ± 0.4(6)	8.4 ± 0.4(9)	8.8 ± 0.4(5)	8.0 ± 0.5(5)[a]	7.7 ± 0.3(6)[d]	$P < 0.01$
	F_{1b}	9.8 ± 0.4(6)	9.9 ± 0.4(6)	9.2 ± 0.4(9)	9.1 ± 0.7(5)	6.4 ± 0.2(6)[c]	6.8 ± 0.2(6)[d]	$P < 0.001$
Day 21	F_{1a}	41.8 ± 1.2(6)	32.9 ± 2.4(6)[b]	36.8 ± 1.5(9)[a]	43.0 ± 2.2(5)[d]	38.8 (1)[d]	40.7 (1)[d]	NS
	F_{1b}	45.2 ± 2.6(5)	35.8 ± 2.5(6)[a]	40.3 ± 2.2(7)	43.2 ± 4.1(5)[d]	—	41.2 ± 2.2(2)[d]	NS

[a] $P < 0.05$.
[b] $P < 0.01$.
[c] $P < 0.001$.
[d] No meaningful comparisons of body weight can be made as substantial mortality occurred.
NS, not significant.

TABLE II

RAT PUP HCB RESIDUE LEVEL (μg/g)

Newborn pups were obtained from dams fed 60 ppm HCB before suckling. Day 21 rat pups were exposed to HCB via maternal milk and from consuming some HCB diet themselves. Data is the mean ± S.E.M. for the indicated number of rat pups.

	F_{1a}	F_{1b}
Newborn pups	8.3 ± 0.4(14)	5.4 ± 0.3[b](6)
Day 21 pup liver	161.0 ± 9.5(17)	150.0 ± 11.3(7)
Day 21 pup brain	68.0 ± 2.4(17)	53.0 ± 3.5[a](12)

[a] $P < 0.01$.
[b] $P < 0.001$.

In light microscopic study of the lungs, 2 types of structural changes were found in the dams treated with HCB for 6 months. Collections of intraalveolar foamy histiocytes were seen more frequently in the treated groups than in control rats. Hypertrophy and proliferation of the lining endothelial cells in pulmonary venules were observed in HCB treated rats. In the lumen of affected vessels an increase in leukocytes (chiefly lymphocytes) was observed.

DISCUSSION

The dietary concentrations used in this study (0, 60, 80, 100, 120, 140 ppm HCB) were carefully selected to bracket the LD_{50}-value for nursing pups. This allows a more accurate determination of the maternal dietary LD_{50} to suckling pups, as the 5 doses used range from slight effects to near total mortality [11]. The suckling period is the most sensitive phase of the life cycle for HCB lethality [5]. In this study the concentrations of HCB in the brains of the F_1 pups of dams fed 60 ppm was 68 and 53 μg/g in the F_{1a} and F_{1b} generations. This dietary concentration is slightly more than half the LD_{50}-value (104 ppm dietary concentration). In contrast, death occurs in adult rats only after the brain HCB concentrations exceed 300 μg/g [2]. The nervous system appears to be the target organ of HCB lethality [12]. De Matteis et al. [13] first noticed the neonatal mortality of HCB to suckling pups by using doses close to those maternally toxic. Subsequent work by Grant et al. [5] found 320 ppm and 640 ppm HCB to be a maternally toxic level, but many nursing pups died from dams fed 160 ppm HCB in the F_1 generations. At a dose of 80 ppm Grant et al. [5] found decreases in the number of pups surviving to 21 days by the F_3 generation.

The neonatal brain levels of HCB found in this study are in fairly good agreement with those observed by Grant et al. [5]. They found 30 μg/g and 33 μg/g HCB in brain of male and female pups nursed from dams fed 80 ppm HCB. At the same dietary concentrations other studies found 41 μg/g HCB in 21 day pup brain [4].

The increased incidence of intraalveolar foamy histiocytes and the changes in the endothelial cells in venules confirm the earlier report of Goldstein et al. [14]. With regard to HCB exposure, the Sherman strain of rat shows a larger increase in intraalveolar foamy histiocytes [15] while the Sprague—Dawley rat is more likely to develop hypertrophy and proliferation of the endothelial cells of the pulmonary venules [14].

Results of this study have accurately determined the LD_{50} of HCB to F_{1a} and F_{1b} generation suckling rat pups to be 100 ppm and 104 ppm (dietary maternal concentration). Thus with mortality as an endpoint no evidence of substantial depletion of maternal body burden of HCB was observed. The comparison of the rat pup HCB residual levels between the F_{1a} and F_{1b} generations indicate a 15—20% reduction in the F_{1b} HCB body burden. In this study litter size was not maintained at a constant level throughout lactation. Thus when substantial neonatal mortality occurred (at 100 ppm HCB and higher in the F_{1a}) less pups were available to reduce maternal body burdens of HCB. Further studies using litters maintained at constant size throughout lactation are required to determine if lactational transfer of HCB to pups decreases the body burden to a level at which intergenerational neonatal mortality differences are evident.

ACKNOWLEDGEMENTS

The authors thank Mr. Keith McElroy, Mr. Robert Thomas, Ms. Lillian Strader for their fine technical assistance.

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